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STUDIES IN INDIAN FIBRE PLANTS

No. 3. ON THE INHERITANCE OF CHARACTERS IN *HIBISCUS SABDARIFFA* L.

BY

ALBERT HOWARD, C.I.E., M.A., A.R.C.S., F.L.S

Imperial Economic Botanist

AND

GABRIELLE L. C. HOWARD, M.A

Second Imperial Economic Botanist

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CONTENTS.

		Page
I.	Introduction	47
II.	Experimental results	49
	1. The presence of red or crimson colour	50
	2. The red patches on the veins, petioles and stems ..	55
	3. The general colour of the stem	60
	(*a*) The crimson factor of var. *ruber*	60
	(*b*) The flushes on the stems of var. *intermedius* and var. *Bhagalpuriensis*	62
	4. The corolla	66
	5. The colour of the calyx	68
	(*a*) The sepal tips	68
	(*b*) The red colour on the sepals	74
III.	Summary	75
IV.	Conclusions	82

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I. INTRODUCTION.

Roselle (*Hibiscus Sabdariffa* L.), known in the vernacular as *mesta, patwa, lal ambari* or *kempu*, is grown for a variety of purposes in many parts of India, Ceylon, and the West Indies. Almost every part of the plant can be utilized. A strong silky fibre—Roselle hemp—is obtained from the stems; the fleshy calyces and leaves are used for food, while the seeds are employed in medicine. The study of this species was begun at Pusa some years ago when four varieties were isolated and described, namely—*ruber, albus, intermedius* and *Bhagalpuriensis*.[1] These four varieties are almost identical morphologically. In var. *Bhagalpuriensis* the calyx is slightly more twisted and obtuse than in the other three varieties. This is the only difference in form, which could be detected. With this minor exception, the sole differences between the varieties lie in the distribution of colour.

Roselle is admirably adapted for the training of students in the technique of plant-breeding. The cultivation is simple, the plants are robust, practically immune to pests and not at all sensitive to water-logging or to defective soil

[1] Howard, A., and Howard, G. L. C. Studies in Indian Fibre Plants, No. 2.—On some new varieties of *Hibiscus cannabinus* L., and *Hibiscus Sabdariffa* L., *Memoirs of the Dept. of Agr. in India* (*Botanical Series*), IV, 1911, p. 35.

aeration. The flowers are large enough for easy manipulation but the experimental plants require constant attention as the flowers open singly. Moreover, this species flowers in November and December between the monsoon and cold season crops when other material suitable for study is scarce. The uniformity in the form and size of the plants enables the student to concentrate on the colour differences. In order to employ Roselle to the best advantage for training purposes, a thorough study of the colour factors was first of all necessary. An investigation into the chemical and physiological factors responsible for these colour differences was also contemplated. Unfortunately, however, the plants require a great deal of space. It is impossible to grow them closer than three feet apart each way. Owing to the expansion, during recent years, of the work on other crops, the pressure on the limited area of land at the disposal of the Botanical Section has been so great that only a very small space could be spared for the Roselle cultures. This circumstance has delayed the work considerably. As the continuation of the investigation is doubtful, we have considered it advisable to publish the results so far obtained. Some of the characters have been worked out in detail, others require further study.

The following general description of the four varieties employed will help to bring out the various differences in the distribution of the colour factors :—

Var. *ruber* (PLATE I).

Stem dark red. *Stipules* dark red. *Leaves* green with some red colour on the lower surface of the veins, sometimes also on the upper surface ; gland on the midrib colourless ; petiole dark red except for a narrow strip on the under surface ; pulvinus red. *Peduncle* red. *Epicalyx* red. *Sepals* red ; central gland on mid-nerve greenish. *Corolla* yellow, with a deep crimson eye, turning a deep salmon pink on withering. *Stamens* staminal tube red ; pollen and anthers deep red. *Stigmas* red. *Seedlings* stem green with some red below the cotyledonary leaves ; petiole red on the upper surface, green below ; leaves green.

Var. *albus* (PLATE II).

Stem green. *Stipules* green. *Leaves* green, no red colour on the veins ; pulvinus green ; petiole green. *Peduncle* green. *Epicalyx* green. *Sepals* yellowish green ; apices yellow when ripe. *Corolla* yellow with colourless eye. *Stamens* staminal tube white showing up the yellow pollen. *Stigmas* white. *Seedlings* stem, petiole and cotyledonary leaves green.

HIBISCUS SABDARIFFA, L. var. RUBER.

HIBISCUS SABDARIFFA L. var. ALBUS.

HIBISCUS SABDARIFFA L. var INTERMEDIUS.

HIBISCUS SABDARIFFA. L. var BHAGALPURIENSIS.

Var. *intermedius* (PLATE III).

Stem green with some diffused reddish colour and with deep red triangular patches both in the axil of the leaves and also immediately below the petiole. *Stipules* green. *Leaves* green with some reddening on the upper surface of the veins; pulvinus red. *Peduncle* green. *Epicalyx* green. *Sepals* yellowish green; apices green when ripe, a red spot in the central gland on the mid-nerve. *Corolla* yellow with a deep crimson eye, remaining yellow on withering. *Stamens* staminal tube red; pollen deep orange. *Stigmas* red. *Seedlings* indistinguishable from those of *ruber*.

Var. *Bhagalpuriensis* (PLATE IV).

Stem green with some diffused red colour and a deep red triangular patch in the axil of the leaves. *Stipules* green. *Leaves* green, veins green; petiole green with a certain amount of diffused redness, pulvinus green. *Peduncle* green. *Epicalyx* green. *Sepal* bright green with splashes of red; central gland with a red spot. *Corolla* yellow with a crimson eye which is less deeply crimson than in *intermedius*, salmon pink when faded. *Stamens* staminal tube red; pollen less deeply orange than in *ruber* and *intermedius*. *Stigmas* red.

All the four varieties are normally self-fertilized and set well under bag. Pollination is, however, occasionally effected by humming birds but this occurs so rarely that natural cross-fertilization can be ignored in practice. As a precautionary measure, the flowers of all the plants used in these investigations with very few exceptions, were protected. Two minor difficulties were encountered in the work, namely, (1) the hardness of the seed coat in *Bhagalpuriensis* and in the hybrids derived therefrom and (2) the dormancy of the seeds left in the soil. The first difficulty was overcome by pricking before sowing the seeds of *Bhagalpuriensis* and of the hybrid cultures in which this variety was one of the parents. This, although very laborious when the number of cultures was large was uniformly successful. The second difficulty can only be partially avoided by a long rotation and by germinating the fallen seeds on the surface of the ground before they are buried by the plough.

II. THE EXPERIMENTAL RESULTS.

The four varieties were all crossed *inter se*, *i.e.*, *intermedius* × *Bhagalpuriensis*, *intermedius* × *ruber*, *intermedius* × *albus*, *Bhagalpuriensis* × *ruber*, *Bhagalpuriensis* × *albus* and *ruber* × *albus*. In addition, a certain number of crosses

were made between the four varieties and some of the new extracted forms. The F_1 generations were always uniform and the reciprocals alike.

1. The presence of red or crimson colour.

As regards the presence of red colour, the four varieties and the homozygotic phenotypes derived from them by hybridization can be divided into three main groups: (1) those in which the general colour of the plant is green with a certain amount of red distributed on various parts in definite patches: these patches may be accompanied, as in *intermedius* and *Bhagalpuriensis*, by very light general flushes—pink or brown—the tone of which is not sufficiently deep to obliterate them, (2) those like *ruber* in which all such markings are covered by a general crimson colouration of the plant, (3) those like *albus* in which no red or crimson colour at all is present.

The first subject investigated was the nature of *albus*. The general green appearance of this variety might be due either to the absence of all the factors responsible for colour in the other varieties—in which case it would be of the greatest use as an analyser—or the colour factors might be present in a suppressed state. In the investigation of the genetic constitution of *albus*, the first cross to be considered is that of *ruber* × *albus*. The general stem colour of *ruber* is crimson. This colour extends over most of the other parts of the plant but the extreme base of the stem is green. This point proved to be of importance in distinguishing true *ruber* from other crimson forms produced by hybridization. The leaves of *ruber* are green with some crimson on the lower surface of the veins. The petiole is crimson except for a narrow strip on the under surface; the peduncle, epicalyx and sepals are all crimson. The staminal tube, pollen, anthers and stigma are also crimson. *Albus* is very different. There is no red colour on any portion of the plant. The general tone is a yellowish green, quite different from the brighter green of *intermedius*. The apices of the sepals are yellow when mature. The staminal tube and stigmas are white, the pollen yellow.

The F_1 between *ruber* and *albus* was crimson and was practically identical with the *ruber* parent. In the F_2 (Table I), a mixture of crimson and green plants was obtained in which the crimson plants resembled *ruber* and the green *albus*. Nothing in any way different from the two parents was produced.

Table I.

The F_2 generation of the cross ruber × albus.

No. of culture		Total No. of plants	Crimson like *ruber*	Green like *albus*	Ratio crimson : green
Ruber × albus	1	300	235	65	..
	2	298	232	66	..
	3	313	235	78	..
	4	505	370	135	..
	5	298	236	62	..
	6	225	180	45	..
Albus × ruber	7	199	154	45	..
	8	230	178	52	..
	9	298	236	62	..
Total		2,666	2,056	610	3·4 : 1
Expectation		....	*1999·5*	*666·5*	....

Table II.

The F_3 generation of the cross ruber × albus.

No. of culture	Colour of parent	F_3		
		Total No. of plants	Crimson	Green
13	green	42	....	42
14	Do.	55	....	55
15	Do.	66	....	66
16	Do.	45	....	45
17	Do.	5	...	5
19	Do.	46	....	46
20	Do.	28	....	28
18	Do.	29	1*	28
Total		....	316	1	315
Expectation		....	....	*0*	*316*

* Probably a natural cross as in this case the flowers of the parent were not protected.

TABLE II—*contd.*

No. of culture	Colour of parent	F_3		
		Total No. of plant	Crimson	Green
1	crimson	40	40	....
6	Do.	38	38	....
11	Do.	11	11	....
TOTAL	....	89	89	0
Expectation	....	....	*89*	*0*
2	crimson	41	22	19
3	Do.	53	39	14
4	Do.	41	29	12
5	Do.	89	66	23
7	Do.	35	25	10
8	Do.	52	44	8
9	Do.	77	50	27
10	Do.	77	58	19
12	Do.	25	18	7
TOTAL	....	490	351	139
Expectation	....	....	*367·5*	*122·5*

The experimental numbers obtained do not agree very closely with expectation but the error is reversed in the two generations.

Similar results were obtained when *albus* was crossed with the two varieties which possess green stems with red markings, *intermedius* and *Bhagalpuriensis*. The F_1 in both cases was predominantly crimson and very like *ruber* although certain differences in detail could be detected. In the F_2,

the following types were found :—(1) green plants without any red at all, (2) green plants with red markings and (3) plants predominantly crimson. The following are the numerical results obtained :—

TABLE III.

The F_2 generation of the crosses intermedius × albus and Bhagalpuriensis × albus.

Cross		Total No. of plants	Plants either crimson or with red markings	Plants entirely green	Ratio crimson : green
Intermedius × albus	A	45	37	8	..
	B	76	58	18	..
	C	85	70	15	..
	D	37	27	10	..
	E	84	59	25	..
	F	66	43	23	..
TOTAL		393	294	99	3 : 1
Expectation		..	*294·5*	*98·5*	
Albus × Bhagalpuriensis	A	82	59	23	..
	B	73	58	15	..
	C	86	59	27	..
	D	78	64	14	..
	E	79	69	10	..
	F	80	59	21	..
Bhagalpuriensis × albus	G	78	54	24	..
	H	74	55	19	..
	I	70	51	19	..
	J	61	44	17	..
	K	95	73	22	..
	L	86	59	27	..
TOTAL		942	704	238	3 : 1
Expectation		..	*706·5*	*235·5*	

Certain of the crimson plants and some of the green plants with red markings again split in the following generation into plants with colour and green plants, the green plants forming approximately one quarter of the whole. The results are given in the following Tables :—

TABLE IV.

Crimson plants giving crimson and green plants in the F_3 generation of the cross Bhagalpuriensis × albus.

No. of culture	Crimson plants	Green plants	Ratio crimson : green
A 6	22	5	
A 7	46	22	
A 23	11	5	
A 25	36	11	
A 30	58	15	
A 40	52	17	
A 42	25	6	
A 43	54	14	
A 55	49	25	
A 61	35	12	
A 66a	58	20	
TOTAL	446	152	2·9 : 1
Expectation	*448·5*	*149·5*	

TABLE V.

Green plants with red markings giving a certain number of entirely green plants in the F_3 of the cross Bhagalpuriensis × albus.

No. of culture	Green plants with red markings	Green plants	Ratio crimson : green
A 3	42	15	
A 14	40	19	
A 12	40	18	
A 15	34	17	
A 63	48	21	
A 64	43	18	
A 59	58	16	
A 71	32	5	
A 80	60	20	
A 75	50	16	
A 76	40	22	
TOTAL	487	187	2·6 : 1
Expectation	*505·5*	*168·5*	

Similar results were obtained in the F_3 of *intermedius* × *albus*. In all the crosses, plants which are entirely green bred true to this character in the succeeding generations. These results point to the absence of a colour producing factor or the presence of a colour inhibiting factor in *albus*. Taking into consideration the relation between anthocyanin and flavone found by many investigators and the fact that the whole appearance of *albus* is yellowish green rather than green, it seems probable that this variety contains the yellow flavone. *Ruber* contains a factor R which converts this into anthocyan. The green plants extracted in the F_3 from the green plants with red markings in the crosses of *albus* with *intermedius* and *Bhagalpuriensis* do not possess the factor for the crimson colour of *ruber* and are therefore green and not yellowish green. These extracted green plants when crossed among themselves or with *albus* gave in the F_1 and succeeding generations green plants only, no coloured plants of any kind being produced. When crossed on to *Bhagalpuriensis* they gave a mixed progeny in the F_2—plants with red markings and plants entirely green. In most cases, the coloured portion of the cultures consisted of individuals with very varied markings on the stems and leaves but in no case were the markings less than those of *Bhagalpuriensis*. When crossed with *ruber*, a mixed progeny of red plants, of green plants with red markings and of entirely green plants were produced in the F_2. It is clear that the extracted greens were merely green plants with red markings in which these markings cannot find expression owing to the absence of the colour producing factor R. In no case has it been possible either to obtain any green plant without X (the factor or group of factors responsible for the markings of *Bhagalpuriensis*) or to break this down into several factors.

2. The red patches on the veins, petioles and stems.

The red or brown colouring of the stem is present in two forms—either as well defined patches of dark red or as flushes or washes with indeterminate outlines which occur over large portions of the stem. The flushes vary in colour and distribution. As this investigation showed that there was no genetic connection between these two systems of colouring, they will be treated separately.

The only two varieties in which the distribution of the isolated patches of colour could be studied were *intermedius* and *Bhagalpuriensis*. The following Table and Plate V show the differences between the two varieties in respect to these characters.

TABLE VI.

Differences in the stem and leaf markings of intermedius and Bhagalpuriensis.

	Intermedius	*Bhagalpuriensis*
Stem	Green with two triangular red patches, one in the leaf axil and one below the petiole	Green with one triangular red patch in the axil of the leaf
Pulvinus	Deep red	Green
Leaf veins	A red spot on all the five primary veins	A red spot on the four outer primary veins only, the central one green

Bhagalpuriensis represents what may be termed the basic form as regard the colouration of the veins, petioles and stems. It has not been found possible to produce by hybridization any plant without the factors for these markings. It is true that perfectly green phenotypes were obtained but, as in the case of *albus*, these proved to be merely potentially coloured forms in which the coloured markings were suppressed. The red triangular patch on the stem in the axil of the leaf is apparently inseparable from the four dots on the veins.

Intermedius × *Bhagalpuriensis.*

In all the characters investigated, the F_1 was uniform and intermediate between the two parents. The seeds of five F_1 plants were sown separately in 1912 and the F_2 in each case consisted of a series of types representing every conceivable gradation between the parents but with nothing greater or less than these. A definite statistical examination of the F_2 proved to be impossible for the following reasons. In the first place, there is a certain amount of variation due to environment and age. As the leaves, stems and petioles grow older they become red all over. Sunlight and shade also effect the intensity and extent of the colour, intense sunlight producing the effect of age. In the second place, the large number of factors involved in so small a phenotypic difference made identification of the various genotypes very difficult. During the course of these investigations, it was found that no less than three factors were concerned in building up the colour markings of *intermedius* from those of *Bhagalpuriensis*. The eight homozygotic phenotypes, resulting from the

RUBER.

INTER.

BAND
HALF-WAY.

RED PATCHES ON THE

BHAG.

ALBUS.

WASH

FIVE DOTS.

EINS, PETIOLES AND STEMS.

interaction of these three factors, together with the large number of heterozygotes produced an almost perfect series in which it was extremely difficult to state, from observation only, to which class any individual plant belonged. The following method was, therefore, adopted. Seed of all the plants (145) of one of the F_2 cultures was sown plant by plant in 1913 and observations were made on the offspring. In the following year, a large number of selected cultures were continued into the F_4 and succeeding generations by growing several plants from each. It was quite easy to recognize homozygous cultures and also cultures in which there was only a difference of one factor. In cultures in which more than one factor were concerned, although it was possible to determine the nature of the culture and the factors involved, it was not possible to assign individual plants to their correct class with any degree of certainty.

By continuing this method to the F_7 generation, it was found that the colour markings of the leaves of *intermedius* are due to the factor or factors present in *Bhagalpuriensis* (which will be termed X in future) plus three factors A, B and W. These three factors are quite independent of one another and can be inherited separately. The presence of A converts a leaf with dots on the four outside veins into one with five dots, *i.e.*, a dot on each principal vein. Cultures breeding true to five dots were obtained and also cultures containing both four dots and five dots. The outward manifestations of B and W are somewhat alike and these two factors were at first confused with one another. More accurate observations, however, showed that they were really quite distinct, and comparatively easy to differentiate. B produces a red band on the petiole below the pulvinus. W also gives rise to some red colour on the petiole, but instead of a definite band with well-marked edges it produces a light red wash of somewhat indeterminate outline. Uniform cultures of both *band* and *wash* types were obtained and also cultures splitting between *Bhagalpuriensis* and the *band* type and *Bhagalpuriensis* and the *wash* type. These factors were also found in combination, *i.e.*, five dots and a band, five dots and wash, four dots and a band and four dots with wash. Finally all three, five dots, band and wash are present in *intermedius*. Uniform cultures of all these phenotypes were obtained and grown for some years. Most of the numerous heterozygotic cultures which should be produced by the interaction of these three factors were also identified and checked by continuing several plants from these cultures to the succeeding generation. The various combinations are shown in Plate V.

The results are summed up in the following Tables :—

TABLE VII.

Homozygotic combinations obtained from the cross Bhagalpuriensis × intermedius.

Genotype	Phenotype	Abbreviated designation
1. (XX)aabbcc	Four dots on the leaf veins, petiole green	*Bhagalpuriensis*
2. (XX)AAbbcc	Five dots on the leaf veins, petiole green	Five dots
3. (XX)aaBBcc	Four dots on the leaf veins, plus a red band on the petiole	Band
4. (XX)aabbWW	Four dots on the leaf veins, plus a red wash on the petiole	Wash
5. (XX)AABBww	Five dots on the leaf veins, plus a red band on the petiole	Band half-way
6. (XX)AAbbWW	Five dots on the leaf veins, plus a red wash on the petiole	Wash half-way
7. (XX)aaBBWW	Four dots on the leaf veins, a red band and a red wash on the petiole, *i.e.*, it resembles *intermedius* except for the absence of a red dot on the central vein	*Intermedius* with four dots
8. (XX)AABBWW	Five dots, a red band, plus a red wash on the petiole	*Intermedius*

The uniformity of these cultures was always tested by selecting several plants at random and growing the seed from them next to each other during the following year.

Cultures splitting between the following limits were also identified and tested :—

TABLE VIII.

Splitting cultures identified in the F_3 and succeeding generations from the cross Bhagalpuriensis × intermedius.

Limits of the culture	Genotype of the parent
1. *Bhagalpuriensis* to five dots	(XX)Aabbww
2. *Bhagalpuriensis* to wash	(XX)aabbWw
3. *Bhagalpuriensis* to band half-way	(XX)AaBbww
4. *Bhagalpuriensis* to wash half-way	(XX)AabbWw
5. *Bhagalpuriensis* to *intermedius*	(XX)AaBbWw
6. Five dots to band half-way	(XX)AABbww
7. Five dots to wash half-way	(XX)AAbbWw
8. Five dots to *intermedius*	(XX)AABbWw
9. Band to band half-way	(XX)AaBBww
10. Band to *intermedius*	(XX)AaBBWw
11. Wash to wash half-way	(XX)AabbWW
12. Wash to *intermedius*	(XX)AaBbWW.
13. Band half-way to *intermedius* with four dots	(XX)AaBBWw
14. Band half-way to *intermedius*	(XX)AABBWw
15. Wash half-way to *intermedius*	(XX)AABbWW
16. *Intermedius* with four dots to *intermedius*	(XX)AaBBWW

These cultures were tested in the same manner as the homozygotic ones, *i.e.*, by growing in the following year several plants from each culture separately. In these cases, instead of selecting the plants at random, care was taken to include plants representing the extreme phenotypes.

A few cultures, which theoretically should have been present, were not found; most of these involve the phenotype *intermedius* with four dots. This form is very difficult to identify and these cultures were probably confused with the corresponding ones in which *intermedius* occurred. The pure culture of *intermedius* with four dots and the splitting cultures—band half-way to *intermedius* with four dots and *intermedius* with four dots to *intermedius*—were, however, identified with certainty. The other cultures were doubtless present and either escaped recognition or did not happen to occur among the plants selected for further investigation.

TABLE IX.

Splitting cultures, theoretically required, which were not found.

Limits of culture	*Genotype of parent*
1. *Bhagalpuriensis* to band	(XX)aaBbww
2. *Bhagalpuriensis to intermedius* with four dots	(XX)aaBbWw
3. Five dots to *intermedius* with four dots	(XX)AABbWw
4. Band to *intermedius* with four dots	(XX)aaBBWw
5. Wash to *intermedius* with four dots	(XX)aaBbWW
6. Wash half-way to *intermedius* with four dots	(XX)AaBbWW

The stem markings in the axil of the leaf and below the petiole are due to the same factors which affect the red colouration of the petiole and leaf-veins. Each factor produces a change in the stem markings as well as on the leaves. The factor or factors which produce the four dots in *Bhagalpuriensis* are also responsible for the triangular red patch in the axil of the leaf. The factor A which controls the fifth dot also produces two narrow red streaks on the stem which start from the two basal angles of the triangle and run a short way down the stem. The factor B not only gives a band across the petiole but longer and larger red streaks on the stem. The factor W produces a certain amount of red colour below the petiole. The presence of all three factors together is shown by the well-marked triangular patch below the petiole in *intermedius*. In no case was it possible by breeding to separate the leaf-

markings from the corresponding stem-markings. We must, therefore, conclude that the difference in the red colouration of the leaf, petiole and stem of *intermedius* from that of *Bhagalpuriensis* is due to three factors only. The presence of each factor is shown by a definite addition to the markings both of the leaf (including the petiole) and of the stem.

3. The general colour of the stem.

It was pointed out in a previous section (p. 50) that in addition to the well defined patches of red on the stem, certain flushes or washes also occur over large portions of the stem. These vary in colour and distribution and may be roughly divided into (1) those like the crimson factor in *ruber* which obscure all other stem markings and which affect other portions of the plant simultaneously and (2) those which are light in colour, which allow the stem markings to be identified and affect the stem only.

(*a*) *The crimson colour factor of ruber.*

The next factor which must be considered is the one which is responsible for producing the red colour over the stem and the major portion of the plant. This crimson flush, as it may be called, has a very far reaching effect and whenever it is present, the whole plant would be classed as crimson or scarlet. In all crimson types there is a certain amount of green, the distribution and amount depending on the genetic constitution of the type. For instance, in the F_1 between *intermedius* and *albus*, the green base of the stem was more extensive than in *ruber* and the lower eighteen inches of the basal branches were also green. Variations in the amount of green in the petioles, leaves, epicalyx and peduncle occur in types which are nevertheless crimson. Whatever the constitution, however, if the stem is red, the branches and sepals are also red and the corolla changes to a deep pink on withering. A red calyx is inseparable from a red stem. The following experiments show that this crimson flush, which converts a green plant with red markings into a crimson one, is produced by a single factor.

The first cross made in this connection was that of *ruber* × *intermedius* *i.e.*, the cross between a crimson type and the green type which possesses the greatest amount of red markings yet observed. The F_1 was crimson and resembled *ruber*, except that more green was present and the crimson colour was slightly less intense. In the F_2, crimson plants and green plants, with the same red markings as those of *intermedius*, were obtained. It was possible to divide the crimson plants into two classes, those which were as deeply crimson as *ruber* and those in which the stem colour was lighter in tone.

The results obtained are given in the following Tables :—

TABLE X.

The F_2 generation of the cross ruber × intermedius.

No. of culture	Total No. of plants	Colour of stem		
		Deep crimson	Crimson	Green with red markings
M	77	21	39	17
N	35	9	14	12
O	47	10	23	14
P	76	22	38	16
TOTAL	235	62	114	59
Ratio		0·95	1·93	1
Expectation		*58·75*	*117·50*	*58·75*

TABLE XI.

The F_3 and F_4 generation of the cross ruber × intermedius.

	No. of culture	Nature of the parent	Nature of the offspring
F_3	M 3	green with red markings	all green with red markings
	M 13	Do.	Do. Do.
	M 44	Do.	Do. Do.
	M 70	Do.	Do. Do.
	M 71	Do.	Do. Do.
	M 1	crimson	10 green with red markings : 26 crimson : 12 deep crimson
	M 2	Do.	10 green with red markings : 21 crimson : 11 deep crimson
	M 10	Do.	green with red markings, crimson, deep crimson
	M 11	Do.	Do. Do.
	M 14	deep crimson	all deep crimson
	M 43	Do.	Do.
	M 51	Do.	Do.
	M 54	Do.	Do.
	M 56	Do.	Do.
F_4	M 1–7	green with red markings	all green with red markings
	M 1–4	crimson	143 deep crimson and crimson 36 green with red markings
	M 1–3	deep crimson	all deep crimson
	M 13–1	green with red markings	65 plants, all green with red markings
	M 14–1	deep crimson	65 plants, all deep crimson

Similar results were obtained in the cross *ruber* × *Bhagalpuriensis*. The F_1 was a crimson plant slightly less deep in colour than *ruber* and differing in various details of the colouring. In the F_2, both crimson plants and green plants with red markings were obtained in the following proportion—97 crimson plants to 28 green plants with red markings, a ratio of 3·4 : 1. Thus the evidence from these two crosses indicates that the crimson flush is produced by a single factor which may be termed S.

It was shown in the last section (p. 55) that *albus* possesses the factor for crimson flush, although, owing to the absence of R, it cannot find expression. The crosses with *albus* should, therefore, also be considered in this connection.

Intermedius × *albus*. The F_1 was crimson and somewhat similar to *ruber*. The F_2 gave 99 colourless plants, *i.e.*, those in which the factor for colour production is absent and 294 coloured. The coloured plants consisted of 208 plants predominantly crimson and 86 green plants with red markings giving a ratio of 2·4 : 1. The green plants bred true to green while the crimson plants and green plants with red markings either bred true or split in various ways.

Bhagalpuriensis × *albus*. Here again the F_1 was crimson and somewhat similar to *ruber*. The F_2 gave 211 colourless plants and 645 coloured. These consisted of 492 predominantly red plants and 153 green plants with red markings, the ratio being 3·2 : 1.

The above investigations point to the existence of a factor S present in *ruber*, which covers the stem, branches, sepals and other parts of the plant with a crimson flush, obscuring all other red markings. This factor is absent in all the other three varieties. If SS or Ss is present, the plant is predominantly crimson.

(*b*) *The flushes on the stems of the cross intermedius* × *Bhagalpuriensis*.

The inheritance of the light flushes which do not obscure the stem markings (*i.e.*, the brown and pink flushes on the stem of *Bhagalpuriensis* and *intermedius*) must now be discussed. These consist of a pink flush in *intermedius* which starts at the base and extends two-thirds up the stem and lower branches and a very slight brown flush on the apices of the stem and branches. In *Bhagalpuriensis* there is a purplish brown flush at the extreme base of the plants and at the apex a flush similar to that of *intermedius* but of a deeper brown and more extensive for it is found over the upper half or third of the stem. The inheritance of these flushes is very difficult to investigate on account of their reaction to changes in the environment, especially to age and sunlight. In the first place, they cannot be seen on young plants but must be

observed on mature individuals. As the plant gets old, however, there is a general reddening of the stem, leaves and petioles which is quite independent of and obscures these definite flushes. In the second place, these flushes will only develop well in a good light. They remain poorly developed in shade. Very intense sunlight, on the other hand, produces the same reddening effect as age. For these reasons, the time during which observations can be made is very short and it has not been possible, up to the present, to investigate these characters fully. There is no doubt, however, that these flushes represent definite characters which are inherited. Preliminary investigations on a large number of crosses between the four original varieties themselves and with extracted homozygotes showed that the occurrence of these flushes is governed by two sets of factors, the one controlling the colour (pink or brown), the other the distribution. Corresponding to the minimum amount of stem markings X, a small light brown apical flush is always present, although the rest of the stem may be pure green. No individual has yet been observed without at least a small apical flush. The factors affecting the colour occur either singly or in combination when new homozygotic phenotypes are produced. Thus in the F_2 of the cross *intermedius* × *Bhagalpuriensis*, not only were plants like the parents obtained but also a series which exceeded the parental limits. The plants in this series varied from individuals without any flush (except the small apical one) to some which were completely covered by a deep purplish brown flush. Plants possessing this deep purplish brown colour over the whole plant bred true to this colour and also to this distribution. Individuals were also found with this colour but only on the upper half, *i.e.*, with the same distribution as that of the apical flush of *Bhagalpuriensis* and a culture was identified which bred true to this deep colour but in which the distribution varied from a complete colouration to a colouration of the upper portion only.

The basal flush of *intermedius* and the apical flush of *Bhagalpuriensis* are complementary to one another in distribution and the presence of both of these would give a completely coloured plant. The purplish brown colour is presumably due to the combination of the pink and brown colours of these two varieties. It seems probable, therefore, that *intermedius* possesses a factor P_1 for pink and *Bhagalpuriensis* another N for brown, the combination P_1N being a purplish brown much deeper than either.

The question of the interaction of these flushes with the scarlet flush S is somewhat difficult to investigate, also the constitution of *ruber* in this respect. There seems no doubt that *ruber* does not contain either the pink flush of *intermedius* or the brown flush of *Bhagalpuriensis*. It appears,

however, to possess a factor controlling a second brown flush which is less purple in tone than that of *Bhagalpuriensis*. Evidence of the absence of the pink flush and the presence of a brown flush was obtained from the cross *ruber* × *intermedius* of which the F_2 was subjected in 1922 to a critical examination. About seventy-five per cent of the plants were predominantly crimson and the remainder consisted of plants with green stems and the red stem and leaf markings characteristic of *intermedius*. The stem flushes on these green stemmed plants varied very considerably both in colour and distribution ; pink, light-brown, dark-brown and various intermediates were present. Certain plants were completely covered by a dark-brown flush. Individuals either with the true pink of *intermedius* or with a dark brown flush were very few in number, the majority had flushes of varying tints of pink and brown, mostly apical. The deep brown flush produced in this cross is quite different in appearance from the purplish brown one obtained in the case of *intermedius* × *Bhagalpuriensis* and the light brown flushes are also much less deep in colour than those of *Bhagalpuriensis*. The variation in the colour of the flushes in the F_2 and the presence of individuals without the pink flush point to the absence of P_1 in the genotype of *ruber*. The presence of individuals with light brown and deep brown flushes indicates the presence of a brown flush M in *ruber*. That this brown flush is not the one present in *Bhagalpuriensis* is indicated by the difference in the tone of the browns. Further evidence on this point is afforded by the cross *ruber* × *Bhagalpuriensis*. Before leaving the discussion of the cross *ruber* × *intermedius* it may, however, be mentioned that the predominantly crimson plants varied in the tone and amount of crimson colour present. This is what would be expected if S were interacting with P_1, M and the factors for distribution.

A consideration of the cross *ruber* × *Bhagalpuriensis* shows that *ruber* does not possess the brown flush of *Bhagalpuriensis*. In the F_2 it was comparatively easy to separate the plants containing SS, *i.e.*, those which would breed true to crimson from those containing Ss which would throw a certain number of plants with green markings. All the cultures were continued into the F_3 when this classification was found to have been correctly made. The SS. plants, however, varied very greatly among themselves both as regards tone and distribution of colour. Some were even more deeply coloured than *ruber* while others were scarlet rather than crimson.

If *ruber* contains S and M and *Bhagalpuriensis* N, we should expect at least four different tones of colour SMn, SMN, SNm and Snm. In addition to these four tones of colour, differences of distribution would have an effect and the number of scarlet or crimson phenotypes breeding true to S would be large.

It was possible to isolate at least three homozygotic forms which were predominantly crimson and which yet were not *ruber*. The first was a form termed *purple* which was almost indistinguishable from *ruber*. The most striking difference was the continuance of the colour to the extreme base of the plant whereas in *ruber* there is a little green at the base. Very careful examination showed that the general colour was very slightly darker than that of *ruber* and this was confirmed by examination of sections under the microscope. The tone of colour by itself was not, however, sufficient to differentiate the two forms by a casual examination. These purple forms bred true or split into a mixture of *ruber* and purple. They were only found when *ruber* or *albus* was crossed with *Bhagalpuriensis*. This purple form must represent therefore the intensification produced by the combination of the flushes in *Bhagalpuriensis* with the flush or flushes of *ruber*. Two other forms were isolated in this cross (*ruber* × *Bhagalpuriensis*) and bred true. These differed from *ruber* in the other direction, the colour was a clearer scarlet and less purple while the amount of green at the base and on the plant generally was much increased. These two forms were termed O and X. In O, the lowest foot of the stem and the lowest foot of the main branches were green with splashes of red. The upper portion of the stem and branches were like *ruber*. The petiole was green at the back, red in front and at the base whereas in *ruber* it is red with a dash of green ; the bracts also were greener. In the form X, there was much more green present : the lower half of the stem and branches were green with red splashes on the branches. These splashes increase in size until the top of the stem and branches are quite red. The petiole is like X but with less red.

The large number of grades in the tone of colour of the crimson plants in the F_2 of this cross, the extraction of homozygotic forms both darker and lighter in stem colour than *ruber* undoubtedly indicate that the stem flush of *Bhagalpuriensis* is not contained in *ruber* but is controlled by an independent factor. The results obtained in the cross *albus* × *Bhagalpuriensis* agree with this.

These preliminary investigations thus indicate the presence of four factors governing the tone of colour of the stem, namely, S a scarlet flush, M a brown flush present in *ruber*, N a brown flush with a purple tinge present in *Bhagalpuriensis* and P_1 a pink flush present in *intermedius*. A good deal more work is required to confirm and finally establish these results.

During the course of these experiments, evidence was also obtained that besides differences in colour, factors governing distribution are involved. Up to the present, it has not been possible to define the factors more accurately

and very large cultures would probably be necessary for the solution of these problems.

4. The corolla.

In all the varieties except *albus*, the corolla is yellow with a crimson eye. Just as it was impossible to obtain any plant without the factor for a certain minimum amount of red colour on the leaves and stem, so it was impossible to produce any type without the factor for the crimson eye. Var. *albus* and other green phenotypes all possess this factor, although it cannot always find expression. There is a slight difference in the depth of eye colour between *Bhagalpuriensis* and *intermedius* but it is so small that a detailed investigation proved to be impossible. In the F_1 of the cross between these two varieties, the eye was as deep a crimson as that of *intermedius*. In the F_2, all the flowers had crimson eyes showing that both varieties possess the same factor for crimson eye and that the difference must be produced by some factor of intensification or the reverse.

There is only one other character of the corolla in which the four varieties differ from one another and that is the colour assumed by the petals while fading. The flowers of this species open late in the morning and close about midday, remaining open for not more than three hours. The closing of the flower is fairly rapid and the petals become twisted after the flowers have closed. During this period, a change takes place in the colour in some varieties, the yellow petals turning salmon pink. This occurs in *Bhagalpuriensis* and in *ruber* but the intensity of the colour is not the same in both. It is much paler in *Bhagalpuriensis*. The petals of *intermedius*, on the other hand, never turn pink. They remain yellow until they wither.

Bhagalpuriensis × *intermedius*.

In the F_1, the corolla on fading turned a very faint pink and was thus intermediate between the parents. The F_2 consisted of a mixture of plants with yellow-fading and pink-fading corollas in approximately the ratio of 1 : 3. Those with pink-fading corollas could be divided into two classes, *i.e.*, those resembling *Bhagalpuriensis* and those resembling the F_1. The plants with yellow-fading corollas invariably bred true to this character in the F_3 and in succeeding generations. Those with pink-fading corollas of the deeper hue also bred true to this character while the more faintly coloured ones gave a mixed progeny of plants with yellow and pink-fading corollas.

TABLE XII.

Inheritance in the F_2 of changes in the corolla colour in the cross Bhagalpuriensis × intermedius.

No. of culture	No. of plants	Corolla on fading pink	Corolla on fading pale pink	Corolla on fading yellow
I	83	24	43	16
II	88	28	45	15
III	144	34	75	35
IV	147	30	88	29
V	128	21	79	28
TOTAL ..	590	137	330	123
RATIO ..	....	1	2·4	·9
Expectation ..	....	*147·5*	*295·0*	*147·5*

That the division into pink and pale pink was not quite accurate is shown by the results of the F_3 generation.

TABLE XIII.

Inheritance in the F_3 of changes in the corolla in the cross Bhagalpuriensis × intermedius.

No. of culture	COLOUR OF COROLLA ON FADING		RATIO
	Parent	Offspring	Pink : yellow
1	yellow	yellow	
2	Do.	Do.	
3	Do.	Do.	
4	Do.	Do.	
5	pale pink	pink, pale pink and yellow	
6	Do.	Do.	
7	Do	Do.	
8	Do.	Do.	
9	Do.	Do.	
10	Do.	Do.	
11	Do.	Do.	
12	Do.	Do.	401 pink to 151
13	Do.	Do.	yellow 2·7 : 1.
14	Do.	Do.	
15	Do.	Do.	
16	Do.	Do.	
17	Do.	pink	
18	pink	Do.	
19	Do.	Do.	
20	Do.	Do.	
21	Do.	Do.	
22	Do.	Do.	
23	Do.	Do.	
24	Do.	pink, pale pink and yellow	

The factor which produces this pink colour in the corolla is also responsible for the red markings in the full grown calyx. In this species, the fruit is covered during the whole period of its development by the fleshy sepals which increase in size with the expansion of the capsule. In both the varieties under discussion, the sepals are green at the apices and at the base and yellowish green to colourless in the middle. In *intermedius*, there is no red colour on the calyx, while in *Bhagalpuriensis* the sepals are splashed with bright red (Plate VI). In the F_2 of the cross between these two varieties, all the plants in which the corolla remained yellow had calyces without any red colouration. This was found to hold in all succeeding generations. The remaining plants of the F_2 all possessed calyces splashed with red but the amount of red colour varied from a few dots to something much more intense than the markings on the capsules of *Bhagalpuriensis*. In all these plants, whatever the amount of red on the calyx, the corolla turned pink on fading. The change in corolla colour is, therefore, associated with the existence of red colour in the calyx. It is not connected either with the amount or with the distribution which are controlled by other factors.

Ruber × *Bhagalpuriensis.*

As stated above, the corolla of both these varieties turns pink but the colour is much deeper in the case of *ruber*. In the F_1 between these two varieties, the faded corolla was pink also. The tone of colour was intermediate between that of the two parents. In the F_2, all the corollas turned pink on fading and the same was the case in the F_3 and succeeding generations. As would be expected from the observations in the cross *intermedius* × *Bhagalpuriensis*, all the calyces in the F_2 were either red or splashed with red. Thus the factor F which is present in *Bhagalpuriensis* and which is responsible for the change in the corolla colour and the production of red markings on the calyces, must also be present in *ruber*. This latter variety, however, possesses some additional factor which produces the intensification of the colour in the fading corolla. The factor F is either absent or suppressed in *intermedius*.

Similar results were obtained in the cross *albus* × *Bhagalpuriensis*. All the green plants with red markings possessed pink fading corollas and calyces splashed with red.

5. The colour of the calyx.

(*a*) *The sepal tips.*[1]

The first character which must be dealt with is the general ground colour of the sepal which is best seen in the mature tips. In *albus*, the tips are yellow,

RUBER

INTER.

BHAG.

ALBUS.

BRIGHT GREEN.

GREEN.

OLIVE GREEN

STRONG FLUSH.

RED DOTS

PINK.

RED

THE COLOURS OF THE MATURE CALYX

in *intermedius*, green, in *Bhagalpuriensis*, bright green. These differences are very distinct and are well shown in Plate VI. The relation between green tips and bright green tips was studied in the cross *intermedius* × *Bhagalpuriensis*. The F_1 was intermediate between the two parents. Beyond ascertaining that the tips of the sepals were of three tones of green, the F_2 was not further investigated. In the F_3 and F_4, however, detailed observations were made in most of the cultures. The results are given in the following Table :—

Table XIV.

Inheritance in the F_3 and F_4 of bright green and green sepal tips in the cross intermedius × Bhagalpuriensis.

A. Cultures which split into green and bright green.

No. of culture	Total No. of plants	Colour of sepal tips		Nature of the red markings on the sepals
		Bright green	Green like *intermedius* and the F_1	
III-3-8	66	16	50	varied from none to markings like those of *Bhagalpuriensis*
III-3-18	66	18	48	like *Bhagalpuriensis*
III-4-3	54	9	45	like *intermedius*
III-4-5	7	1	6	Do.
III-4-14	4	1	3	Do.
III-4-24	18	7	11	Do.
III-6-29	60	11	49	varied from none to markings like those of *Bhagalpuriensis*
III-7-2	34	6	28	varied from markings like *Bhagalpuriensis* to something more intense
III-13-4	27	7	20	varied from markings like those of *Bhagalpuriensis* to something more intense
III-13-7	28	6	22	like *Bhagalpuriensis*
III-13-21	27	7	20	all more intense than those of *Bhagalpuriensis*
III-13-25a	20	5	15	Do.
III-13-43	26	6	20	all more intense than those of *Bhagalpuriensis*
III-13-1	25	6	19	varied from markings of *Bhagalpuriensis* to something more intense
Total	462	106	356	
Ratio		1	3·3	
Expectation		*115·5*	*346·5*	

B. Cultures which bred true in the F_3 and F_4 to green and bright green.

CULTURE	F_3 GENERATION			F_4 GENERATION	
	Total No. of plants	Red markings on the sepals	Colour of the sepal tips	No. of cultures grown	Colour of the sepal tips
III–4–2	39	almost colourless	all green	4	all green
III–4–48	14	like those of *intermedius*	Do.	2	both green
III–5–28	17	all more intense than those of *Bhagalpuriensis*	Do.	2	Do.
III–5–32	?	Do.	all green	3	all green
III–5–30	7	Do.	Do.	–	—
III–6–30	34	Do.	Do.	4	all green
III–7–4	33	like those of *Bhagalpuriensis*	Do.	3	Do.
III–10–36	32	like those of *intermedius*	Do.	–	—
III–10–34	28	Do.	Do.	–	—
III–10–42	29	Do.	Do.	2	all green
III–10–44	28	Do.	Do.	–	—
III–10–78	31	Do.	Do.	–	—
III–13–3	35	varied from none to markings like those of *Bhagalpuriensis*	Do.	–	—
III–3–22	66	Do.	all bright green	–	—
III–6–30	34	more intense than those of *Bhagalpuriensis*	Do.	2	all bright green
III–6–31	33	like those of *intermedius*	Do.	–	
III–7–3	25	Do.	Do.	4	all bright green
III–9–1	34	colourless	Do.	–	Do.
III–9–12	25	Do.	Do.	3	Do.
III–9–13	29	Do.	Do.	5	Do.
III–9–39	22	Do.	Do.	2	Do.
III–9–83	37	like those of *intermedius*	Do.	2	Do.

It will be seen from this Table that bright green behaves as a simple allelomorph to green. It is also clear that the shade of green in the tip is quite independent of the red markings on the sepal. It is possible to get the red markings of *Bhagalpuriensis* combined with the green tips of *intermedius* and vice versa. Two interpretations are possible. We may assume that the green of the sepal tips in *intermedius* is the normal and that the bright green tips are produced by the addition of a factor G. On the other hand, the bright green tips may be normal and the more yellowish green tips of *intermedius* may be produced by a colour restraining factor I which is only present in the flower and capsule and does not affect the rest of the plant. Taking into consideration the fact that the bright green of the sepal tips in *Bhagalpuriensis* is the same shade as that of the epicalyx and of the plant in general, the latter seems the more probable explanation.

The cross *intermedius* × *albus* is an example of green tips × yellow tips but the investigation is somewhat complicated by the red factor which is latent in *albus*. As stated on page 53, the F_1 is crimson and the F_2 a mixture of crimson plants, green plants with red markings and green plants with no red colour. Observations on the crimson plants as regards this character are, of course, impossible. All the green plants with red markings had sepals with green tips like *intermedius*, while the sepal tips in the plants with no red colour were either green, yellow or a shade of green intermediate between these. The sepals with yellow tips formed about one-quarter of the whole number—ratio 1 : 2·7. In the F_3, cultures breeding true to green tips and to yellow tips were found : also cultures which again split into all three forms. The yellow sepal tips are only to be found among plants with no red colour (*i.e.*, those in which R is absent) ; the green plants with red markings all had green sepal tips. Further observations in subsequent generations confirmed this point. The factor which produces yellow tips is therefore dependent on the absence of R.

Further information regarding the constitution of the yellow sepal tips was obtained from the cross *Bhagalpuriensis* × *albus*. Here again yellow tips were only found on plants which were entirely green. In the plants with red markings, the sepal tips were either green, bright green or a shade intermediate to these. In the F_3 and F_4, four homozygous phenotypes were obtained from the entirely green plants, *i.e.*, bright green tips, green tips, yellow tips and a new form with olive green tips. Cultures splitting between these limits were also found. The detailed results as regards sepal tips of all the entirely green plants (22) produced in the F_2 of this cross are given in Table XV.

TABLE XV.

Inheritance of sepal tip colour in the F_3 and F_4 of the cross Bhagalpuriensis × albus.

F_3 GENERATION 1913–14		F_4 GENERATION 1914–15		
			Colour of the sepal tips	
No. of culture	Colour of the sepal tips	No. of culture	Parent	Offspring
A5	14 bright green	A5–1	bright green	all bright green
	26 intermediate	A5–2	olive green	all olive green
	12 olive green	A5–20	intermediate	split bright green to olive green: out of 18 plants 5 were bright green
A10	yellow tips to olive green tips 5 olive green plants in 17	A10–3	olive green	all olive green
		A10–4	intermediate	split into yellow, olive green and intermediate
		A10–5	olive green	all olive green
A11	7 yellow: 14 intermediate: 7 olive green	A11–2	intermediate	3 olive green 10 (F_2 + yellow)
		A11–5	olive green	all olive green
		A11–19	yellow	all yellow
A19	all olive green	A19–1	olive green	all olive green
		A19–2	Do.	Do.
A28	all green	A28–1	green	all green
		A28–2	Do.	Do.
A35	all bright green	A35–1	bright green	all bright green
		A35–2	Do.	Do.
A39	splitting culture containing among other types both bright green and yellow	A39–8	bright green	all bright green except 1 red plant probably a cross
		A39–21	yellow	all yellow

TABLE XV.—*contd.*

F_3 GENERATION 1913–14		F_4 GENERATION 1914–15		
			Colour of the sepals tips	
No. of culture	Colour of the sepal tips	No. of culture	Parent	Offspring
A45	all olive green	A45–1	olive green	all olive green
		A45–2	Do.	Do.
A48	all yellow ..	A48–1	yellow	all yellow
A51	Splitting culture containing yellow, bright green, olive green and green	A51–3	intermediate	splitting culture, 4 olive green : 9 intermediate : 5 yellow
		A51–4	yellow	all yellow
		A51–7	bright green	all bright green
A83	all green	A83–1	green	all green
A58	all olive green	A58–1	olive green	all olive green
A79	all yellow	A79–1	yellow	all yellow
A13	splitting culture	....	....	....
A22	splitting culture	....	....	....
A24	splitting culture	....	....	....
A31	splitting culture	....	....	....
A49	splitting culture	....	....	....
A54	splitting culture	....	....	....
A72	splitting culture	....	....	....
A78	splitting culture	....	....	....
A82	splitting culture	....	....	....

Out of 22 cultures, the following bred true, 1 bright green, 3 olive green, 2 green and 2 yellow. Cultures splitting from yellow to olive green and giving a ratio of approximately 3 : 1 were obtained and a similar culture splitting from bright green to olive green. Cultures containing bright green, yellow, olive green, green and various intermediates were also identified. These results show that in the production of yellow tips another factor

must be involved in addition to I, the factor which reduces bright green tips to green tips. The olive green tips are due to the action of this factor (which may be termed Y) on bright green tips. If we denote the factor or group of factors which is responsible for the bright green of the sepal tips G, the following combinations would be obtained :—

Bright green	GGiiyy
Green	GGIIyy
Yellow	GGIIYY
Olive green	GGiiYY

Yellow or olive green sepal tips are, however, only found in entirely green plants, *i.e.*, in the absence of R. Yellow tips are also characteristic of *albus* which was shown to be a crimson plant in which the colour was not developed, *i.e.*, in which S. is present and R absent. It is possible that YY is, therefore, nothing more than the combination SSrr. This would explain the 3 : 1 ratio. The green plants occurring in the F_2 of crosses with *albus* must contain this factor SS in the following proportions 1 SS : 2 Ss : 1 ss. The various sepal tips would, therefore, be represented by the following combinations :—

Bright green	GGiissrr
Green	GGIIssrr
Yellow	GGIISSrr
Olive green	GGiiSSrr

The combination GGiissRR would also be bright green and GGIIssRR would be green while GGIISSRR and GGiiSSRR would be crimson.

(*b*) *The red colour on the sepals.*

In addition to the changes in the tone of green, the capsules of the four varieties differ very markedly in the amount of red colour on the sepals. The capsules of *ruber* are crimson all over, those of *Bhagalpuriensis* have red splashes on the upper portion, while those of *albus* and *intermedius* appear, at first sight, both to be entirely green. A closer examination shows that this is only true of *albus*. The sepals of *intermedius* have a flush of very faint pink dots on the lower portion of the sepal (Plate VI). As would be expected, no flush is found in plants with absolutely green stems, *i.e.*, those in which R is absent. Plants with red markings on the stem always have some red on the sepals even though it may only be a slight flush as in *intermedius*. As shown before in section 4 (p. 66) distinct red markings on the sepals are always associated with a salmon pink colour of the faded corolla. When the sepals show only the flush, the corolla remains yellow on withering. Neither the red

markings nor the flush, however, show any connection with the amount of red on the stem nor with the tone of green of the sepals (p. 69). It has not been possible to work out in detail the factors governing the nature of the red markings on the sepals but they seem to be governed by factors regulating both the colour and the distribution as was the case with the flushes on the stem. In the F_2 of *intermedius* (green except for faint pink dots on the lower part of the calyx) $\times$ *Bhagalpuriensis* (red markings on the upper portion) a very large number of forms are obtained, many of them much more heavily marked than *Bhagalpuriensis*. On certain plants, the sepals are so heavily marked with red that the whole fruit is almost covered (Plate VI). These forms breed true in succeeding generations. They show two different tones of colour: (1) pink like the flush on *intermedius* and (2) red like the markings of *Bhagalpuriensis*. No calyces are found without any colour but some appear to have a fainter flush even than *intermedius*. *Intermedius* would thus appear to possess a factor for pink which is prevented from expression owing to the absence of F, the factor which produces the colour of the sepals and fading corolla. *Bhagalpuriensis* contains another factor which produces the red tone. *Intermedius* also possesses a pink stem flush but the connection (if any) between the pink colour on the capsule and the pink colour on the stem has not yet been worked out.

In addition to these two factors for colour, there must be factors controlling the distribution. The colour on *Bhagalpuriensis* is confined entirely to the upper half of the sepals, the faint pink dots on *intermedius* occur only on the lower portion, but the more highly coloured fruits extracted from the F_2 are almost completely coloured. The application of the colour also seems to vary. It may occur as distinct dots or as larger splashes. This difference is well shown in Plate VI. There was a certain amount of variation in the distribution of the dots. In some, these were only found on the upper portion of the capsule, as in *Bhagalpuriensis*, but there were no splashes of continuous colour such as occur in that variety. In addition, individuals were found with a stronger pink flush than in *intermedius* (Plate VI). As the disentangling of these factors necessitated more time and land than we have had at our disposal recently, or are likely to have in the near future, it seems best to record these observations now.

III. SUMMARY.

For the sake of clearness, the results obtained by crossing the four varieties may be given in a general form. This will enable some idea to be formed of the genetic constitution of these varieties.

1. *Intermedius* × *Bhagalpuriensis.*

BHAGALPURIENSIS.

Stem. Green with a red triangle above the petiole, brown flush over the upper half of the plant and over the base.

Leaf. Four dots on the back of the outer veins on the underside.

Corolla. Pink-fading.

Sepals. Tips bright green, red markings (splashes and dots) on the upper portion of the mature sepal.

INTERMEDIUS.

Stem. Green with two red triangles, one above and one below the petiole, a pink flush on the lower two thirds of the stem, a very slight brown flush at the apex.

Leaf. Five dots on the veins on the under side; a band of red below the pulvinus, the pulvinus red.

Corolla. Yellow-fading.

Sepals. Tips green, very faint pink dots on the lower portion of the mature sepals.

F_1.

Intermediate in all respects between the two parents.

F_2.

Stem and leaf. All the plants were green with red markings. Nothing was produced outside the limits of the parents. Six intermediate homozygotic forms were found due to the addition of the factors A (fifth dot), B (red band below the pulvinus) and W (red wash over the pulvinus) to X the factor or group of factors present in *Bhagalpuriensis.* These three factors which are quite independent of all other colour factors, each control a certain amount of red colour below the petiole and together form the red triangle present in *intermedius.* As regards the coloured flushes, a slight brown apical flush was observed in all plants derived from this cross. If this apical flush be left out of account, several plants had clean green stems, others were completely covered by a dark purplish brown flush which was shown to breed true. This represents the union of the pink flush of *intermedius* with the brown flush of *Bhagalpuriensis.* These results would point to the existence in *intermedius* of one factor for the pink colour P_1 and another for the distribution and in *Bhagalpuriensis* one factor for the colour N and one or two factors for the distribution.

Corolla. A mixture of pink, pale pink and yellow-fading corollas were obtained in the proportion of 1 : 2 : 1 approximately. Of these, the first and the third bred true, the rest split as before. This change in colour of the corolla on fading was always associated with the presence of colour on the mature calyx but was independent of the amount and also of the stem markings and stem flushes. The presence of red colour in the mature sepals and consequently the pink of the faded corolla are controlled by a single factor F.

Sepals. The colour of the tips varied from green to bright green, the numbers indicating a difference of one factor. Thus if bright green be G, green can be represented by GI.

As regards the colour on the mature capsules, these varied from faint pink dots below like those of *intermedius* or less to a colouration of almost the whole calyx the tone of colour being both pink and red. It is suggested that *intermedius* possesses a factor P for pink plus a factor T for the expression of the colour on the lower half of the calyx while *Bhagalpuriensis* possesses a factor D for red and a factor Q for distribution on the upper half of the calyx. Other factors regulating the appearance of the colour as dots (L) or as splashes (K) are probably also present.

A tentative genetic constitution for *Bhagalpuriensis* would therefore be :—RsFXabwp$_1$mNGipDtQLK plus factors for distribution of the flushes on the stem. For *intermedius* the following is indicated :—RsfXABWP$_1$ mnGJPdTqLk plus factors for distribution of the stem flushes.

2. *Ruber* × *intermedius.*

Ruber.

Plants with a crimson flush over stem, petiole, leaf-veins, peduncle, epicalyx and calyx. A little green at the base of the stem. Corolla fades a deeper pink than in *Bhagalpuriensis.*

F_1.

This is also predominantly crimson (slightly less intense than in *ruber*) but the base and the lower eighteen inches of the branches are green with red splashes ; the leaf-veins, peduncle and epicalyx have splashes of green ; the calyx is crimson but it is slightly less intense in colour than in *ruber.*

F_2.

Crimson plants and green plants with red markings on the stem and leaves were obtained in the proportion of 3 : 1. As regards the red markings

on the stem and leaves, the colour of the sepal tips, the faint pink flush on the sepals and the colour of the corolla on fading, the extracted green plants were exactly similar to *intermedius*. Differences were, however, observed as regards the stem flushes. These varied from pink to brown but nearly all the plants had a decided flush at the apex. *Ruber* possesses a brown flush M (probably apical) which is not present in *intermedius*. As regards the crimson plants, these varied in the distribution of the colour. About one-third bred true to red, the others split giving crimson plants and green plants with red markings in the proportion of 3 : 1.

Ruber would thus appear to have the same constitution as *intermedius* except as regards the factors governing the stem flushes. *Ruber* lacks the factor for the pink stem flush of *intermedius* but possesses the factor S which produces a crimson colour over the stem and the whole of the calyx and also possesses a factor M for a brown stem flush.

3. *Ruber* × *albus*.

Albus.

The plants are entirely green but of a yellowish tone. The sepal tips are yellow. There is no red colour on any part of the plant, even the eye of the corolla is colourless and the pollen is yellow.

The F_1 between these two varieties is indistinguishable from *ruber*. In the F_2, plants resembling *ruber* and *albus* were produced in the proportion of 3 : 1. In the F_3 and succeeding generations, the green plants gave only green offspring, some of the crimson plants bred true, the others split into crimson : green : : 3 : 1. Nothing different to *ruber* and *albus* was produced in this cross. All the green plants had yellow sepal tips.

4. *Albus* × *intermedius*.

F_1.

This resembles the F_1 between *ruber* and *intermedius*.

F_2.

Three classes of plants were produced, the first consisted of crimson plants, the second of green plants with red markings and the third of plants which were entirely green. These formed about one quarter of the whole. The green plants with red markings formed one quarter of the remainder.

The green plants bred true to green in succeeding generations. They were all alike except as regards the sepal tips which split from green to yellow, the

numerical proportion indicating the difference of one factor. This factor YY is probably nothing more than the combination SSrr. The mature sepals were all devoid of the faint pink dots of *intermedius*.

The green plants with red markings resembled *intermedius* exactly except for the stem flushes. The sepal tips were all green and the mature sepals all had the faint pink dots of *intermedius*. In the next generation, about one-third of these plants bred true to everything except stem flushes ; the rest split into plants with red markings and entirely green plants in the proportion of 3 : 1. The entirely green plants produced by the splitting of some of the green individuals with red markings all had green sepal tips. This is in agreement with the hypothesis that the yellow tips are produced when S is present and R absent. Unfortunately, the nature of the stem flushes on the green plants with red markings was not noted. Among the crimson plants some bred true to crimson ; some split again into three classes ; some gave only crimson plants and green plants with red markings (3 : 1), and some gave crimson plants and entirely green ones in the proportion of 3 : 1. As regards the coloured plants, this cross behaved exactly like *ruber* × *intermedius*.

From the results of these two crosses we may conclude that *albus* has the same constitution as *ruber* except for the absence of a factor RR which is necessary for the production of any red colour. Thus

albus plus RR = ruber.

5. *Ruber* × *Bhagalpuriensis*.

F_1.

This was predominantly crimson but with a greater amount of green than in the F_1 of *ruber* × *intermedius*.

F_2.

Crimson plants and green plants with red markings were obtained in the proportion of 3 : 1.

Green plants with red markings.

Stems and leaves. As regards these characters, the plants were not uniform as in *ruber* × *intermedius* but showed all gradations between the markings of *Bhagalpuriensis* and those of *intermedius*. *Ruber* must therefore contain the factors A, B and W which convert the markings of

Bhagalpuriensis into those of *intermedius*. This confirms the results obtained in the cross *ruber* × *intermedius*. The flushes on the stem varied from no flush to a dark brown flush which bred true. This probably represents the union of the brown flush present in *ruber* with the brown flushes of *Bhagalpuriensis*. The tone of this dark brown flush is quite distinct from that of the purplish brown flush produced by the union of the pink flush of *intermedius* with the brown flush of *Bhagalpuriensis*. The presence of this dark brown flush and the occurrence of some plants with no flush show that the brown flush of *ruber* is governed by a different factor to that controlling the brown flush of *Bhagalpuriensis*.

Corolla. In all plants, the corolla turned pink on withering showing that both parents possess the factor F for colour production in the calyx.

Sepals. The tips varied from bright green to green indicating the presence in *ruber* of the factor I. The red markings on the mature sepals varied from something less than the markings of *Bhagalpuriensis* to an almost complete colouration of the calyx and both the pink and dark red colours were present. Some of the least highly coloured calyces showed the red colour in dots only. This would indicate the absence in *ruber* of the factor which converts dots into splashes, and the presence of the factor for pink colour.

The fact that in the F_2 all the mature calyces have red markings point to F being present in both *ruber* and *Bhagalpuriensis*. On the other hand, in the cross *ruber* × *intermedius* only calyces without red markings (except for the faint pink dots) were produced. The scarlet stem flush SS is, however, unlike the other stem flushes, never found without scarlet capsules and the simplest explanation would seem to be a complete linkage of S + F whenever both are present together.

Crimson plants.

The 97 plants comprised in this class differed among themselves very widely as regards the distribution of the crimson colour and the tone of the colour. All (except 3 which were too feeble to give seed) were grown in the F_3. Thirty-four plants gave offspring which were all predominantly crimson, sixty split into crimson plants and green plants with red markings. These thirty-four plants varied among themselves as to the amount of crimson colour present. A certain number were more completely coloured even than *ruber* (especially towards the base) and the colour was a somewhat deeper purple. Some of these cultures bred true in succeeding generations, others split between this purple form and *ruber*. When this extracted purple form was crossed on to *ruber*, only red plants like the two parents were produced.

When crossed on to *Bhagalpuriensis* and *intermedius*, the purple form behaved exactly like *ruber* as regards the factor S. The purple form represents the union of *ruber* with the brown flush of *Bhagalpuriensis*. Thus if the stem flushes of ruber be represented by SM, the purple form would be SMN, where N is the brown flush of *Bhagalpuriensis*.

Another crimson form was identified and found to breed true in succeeding generations. This was slightly less crimson and more scarlet in tone than *ruber* and the lower foot of the stem was green. Indications were obtained of other homozygotic crimson forms depending on the distribution factors connected with these flushes and a pure scarlet form has been identified but has not yet been proved to be homozygous.

6. *Albus* × *Bhagalpuriensis*.

F_1.

The F_1 was crimson but with more green than *ruber* and resembled the F_1 of *ruber* × *Bhagalpuriensis*.

F_2.

Three classes of plants were obtained, crimson plants, green plants with red markings and entirely green plants. The green plants formed about one quarter of the whole, the green plants with red markings one quarter of the remainder. All the F_2 plants were continued into the F_3.

Plants entirely green.

These proved to be homozygous to this character. They differed only in the colour of the sepal tips which were bright green (GGiissRR), green (GGIissRR), olive green (GGiiSSrr), yellow (GGIISSrr) or intermediate between these four homozygotic forms. Several plants with bright green and green sepal tips were crossed on to *intermedius* and *Bhagalpuriensis*. The F_2 gave entirely green plants and green plants with various amounts of red markings showing that the extracted green plants consisted of green plants with red markings in which RR was absent. No scarlet plants were obtained thus confirming the conclusion that yellow sepal tips denote the presence of S, the factor for scarlet. These extracted green plants when crossed among themselves or with *albus* gave, as would be expected, only green plants in the F_2.

Green plants with red markings.

Stem and leaf markings. These varied from those of *Bhagalpuriensis* to those of *intermedius*. Intermediates were found but nothing which could not

be represented by the factors X, A, B and W. Certain individuals in the F_3 produced only green plants with red markings, the others split giving some entirely green plants (3 : 1).

Corolla. In all the plants, the corolla turned pink on fading.

Sepals. The tips varied from bright green to green, indicating the presence in *albus* of the factor I. The mature sepals all had red markings. These markings varied from something less than those of *Bhagalpuriensis* to calyces which were almost completely coloured. The observations on the calyces were made at an early stage of the investigation and fuller details are unfortunately not available. As far as the notes go, the calyces seem to have resembled in their markings those obtained in the last cross.

Crimson plants.

Some bred true to crimson, others split into crimson and green (3 : 1), some into crimson and green with red markings (3 : 1) while others reproduced the F_2 generation. These crimson plants varied very considerably but could not be further analysed. The presence of the purple form produced by the union of *ruber* plus the brown flush of *Bhagalpuriensis* was, however, demonstrated.

IV. CONCLUSIONS.

The investigation of the factors governing the inheritance of the colours in *Hibiscus Sabdariffa*, although incomplete, appears to justify the following conclusions :—

1. There exists a factor R which is necessary for the expression of red or crimson colour in any part of the plant. Colour is also produced in the presence of Rr. This factor is absent in *albus*.

2. The presence of R permits the production of red colour in the stem, leaves, corolla eye and pollen. The production of red colour on the mature calyx and in the fading corolla is, however, dependent on another factor F. This factor only affects the calyx and corolla. It is absent in *intermedius* but present in *ruber* and *Bhagalpuriensis*.

3. The red markings on the underside of the leaf and on the stem (both in the axil of the leaves and below the petiole) are controlled by the same factors.

4. The factor or group of factors X responsible for the red markings in *Bhagalpuriensis* (four dots on the outer veins of the leaf and a red triangle above the petiole) occur in all the four varieties. It has not been possible to produce by hybridization a phenotype without these.

5. Three independent factors A (dot on the fifth vein), B (band below the petiole) and W (red wash on the petiole) are combined with X in the markings of *intermedius*. In addition to their effect on the leaf and petiole, each of these factors is responsible for some red on the stem below the petiole. All three together produce the lower red triangle on the stem.

6. In addition to the red markings, continuous flushes of colour are present on the stem. These flushes vary in colour and in distribution. The most conspicuous is the scarlet flush found in *ruber* which is governed by the factor S. This scarlet flush is always associated with scarlet calyces and pink fading corollas, *i.e.*, S is always linked with F. The distribution of S varies but the crimson colour is never found at the base only. The crimson colour of *ruber* is produced by the presence of a brown flush M (which can exist independently) as well as S. In addition to these two flushes, two others exist, a pink flush in *intermedius* controlled by the factor P_1 and a brown flush N in *Bhagalpuriensis*. The union of P_1 and N produces a purplish brown flush, the union of M and N a dark brown flush, the union of *ruber* (*i.e.*, SM) and N produces a purple form slightly darker than *ruber*. Although both M and N are brown they are not alike. In addition to the factors controlling the colour, these flushes are influenced by distribution factors which may in some cases be linked with the colour but this point has not been sufficiently investigated.

7. The differences in the tone of colour of the sepal tips has been investigated. Representing the bright green of the sepal tips of *Bhagalpuriensis* by G, the green of *intermedius* is due to the addition of a factor I. The yellow colour typical of *albus* has been shown to occur only when S is present and R is absent and is represented by the genotype GGIISSrr. Another homozygous tone of colour, olive green was produced during the course of the investigations. This represents the genotype GGiiSSrr.

8. The factors governing the colour on the mature calyces has not been fully investigated. There appear to be two factors for colour P representing the pink present in *intermedius* (although this is only faintly visible owing to the absence of F) and a factor D present in *Bhagalpuriensis* which converts this pink into dark red. Distribution factors, independent of the colour factors, also occur, *intermedius* probably possessing a factor T for the presence of the colour on the lower half of the capsule, *Bhagalpuriensis* a factor Q for the occurrence of colour on the upper portion only. It is possible that in the future these factors may be proved not to be simple but a group of several factors. In addition, factors are present for the mode of occurrence of the colours, the factor L present in *intermedius* producing

dots. *Bhagalpuriensis* probably possesses a factor K which converts these dots into continuous splashes or produces splashes in addition to dots.

9. The following is the list of factors suggested by these investigations :—

R a factor which is necessary for the expression of all red colour.

F a factor which allows the production of red markings on the calyx and of pink in the fading corolla.

X a factor or group of factors responsible for the red markings of the leaf and the stem in *Bhagalpuriensis*.

A a factor producing a red dot on the central vein of the leaf.

B a factor producing a red band on the petiole below the pulvinus.

W a factor producing a red wash on the pulvinus.

P_1 a factor for the pink stem flush in *intermedius*.

M a factor for the brown stem flush present in *ruber*.

N a factor for the brown stem flush present in *Bhagalpuriensis*.

S a factor for the scarlet stem flush present in *ruber*.

G a factor responsible for the bright green of the sepal tips in *Bhagalpuriensis*.

I a factor which converts bright green sepal tips into the green tips of *intermedius*.

P a factor producing pink markings on the mature calyx.

D a factor responsible for the dark red colour on the calyx of *Bhagalpuriensis*.

T a factor controlling the distribution of red colour on the lower part of the mature calyx.

Q a factor controlling the distribution of the red colour on the upper part of the mature calyx.

L a factor present in *intermedius* by which the red colour on the mature calyx appears in the form of dots.

K a factor which is present in *Bhagalpuriensis* by which the red colour on the mature calyx appears in the form of splashes.

10. From the results of hybridizing the four varieties of *Hibiscus Sabdariffa* among themselves they would appear to possess the following genetic constitutions :—

Bhagalpuriensis	RsFXabwp$_1$mNGipDtQLK.
Intermedius	RsFXABWP$_1$mnGIPdTqLk.
Ruber	R(S+F)XABWp$_1$MnGIPdTqLk.
Albus	r(S+F)XABWp$_1$MnGIPdTqLk.

All probably also contain factors regulating the distribution of the stem flushes.

It is hoped that the publication of these results, although incomplete, may be of use to future workers on this species.

PUSA:

9th November, 1923.

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The following are the publications of the last two years :—

Scientific Reports of the Agricultural Research Institute, Pusa (including the Reports of the Imperial Dairy Expert and Secretary, Sugar Bureau), for the year 1921-22. Price, As. 14.

Scientific Reports of the Agricultural Research Institute, Pusa (including the Reports of the Imperial Dairy Expert and Secretary, Sugar Bureau), for the year 1922-23. Price, R. 1.

AGRICULTURAL PUBLICATIONS—(*concld.*)

Review of Agricultural Operations in India, 1921-22. Price, R. 1-4.

Review of Agricultural Operations in India, 1922-23. Price, R. 1-10.

Proceedings of the Board of Agriculture in India, held at Bangalore on 21st January, 1924, and following days (with appendices). Price, R. 1.

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